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Lipolytic Effects of B-Type Natriuretic Peptide$_{1–32}$ in Adipose Tissue of Heart Failure Patients Compared With Healthy Controls

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Objectives Our goal was to examine the role of B-type natriuretic peptide (BNP) in lipolysis regulation in heart failure (HF) patients.

Background Enhanced adipose tissue lipolysis can contribute to myocardial lipid overload, insulin resistance, and cachexia in advanced HF. Natriuretic peptides were recently recognized to stimulate lipolysis in healthy subjects.

Methods Ten nondiabetic HF patients (New York Heart Association functional class III, 50% nonischemic etiology) and 13 healthy subjects (control subjects) of similar age, sex, and body composition underwent a microdialysis study of subcutaneous abdominal adipose tissue. Four microdialysis probes were simultaneously perfused with 0.1 μM $BNP_{1–32}$, 10 μM $BNP_{1–32}$, 10 μM norepinephrine (NE) or Ringer's solution. Outgoing dialysate glycerol concentration (DGC) was measured as an index of lipolysis.

Results Spontaneous lipolysis was higher in HF patients compared with control subjects (DGC: 189 $\pm$ 37 μmol/l vs. 152 $\pm$ 35 μmol/l, $p < 0.01$). Response to NE was similar ($p = 0.35$) in HF patients and control subjects (DGC increase of 1.7 $\pm$ 0.2-fold vs. 1.7 $\pm$ 0.4-fold). $BNP_{1–32}$ 10 μM markedly increased lipolysis in both HF patients and control subjects (DGC increase of 2.8 $\pm$ 0.5-fold vs. 3.2 $\pm$ 0.3-fold), whereas the response to 0.1 μM $BNP_{1–32}$ was more pronounced in HF patients ($p = 0.02$). In HF patients, spontaneous lipolysis positively correlated with insulin resistance and the response to $BNP_{1–32}$ negatively correlated with adiposity.

Conclusions $BNP_{1–32}$ exerts strong lipolytic effects in humans. Despite marked elevation of plasma immunoreactive BNP, the responsiveness of adipose tissue to $BNP_{1–32}$ is not attenuated in HF, possibly reflecting a deficiency of endogenous bioactive BNP. Lipolytic effects of BNP can contribute to excessive fatty acid mobilization in advanced HF. (J Am Coll Cardiol 2011;58:1119–25) © 2011 by the American College of Cardiology Foundation

A chronic heart failure (HF) state triggers a neuroendocrine response that stimulates the release of free fatty acids (FFAs) from adipose tissue (AT) (1). Although FFAs are the major substrate for cardiac metabolism, persistently increased FFA efflux from AT might have adverse effects on cardiac function (2) by impairing insulin sensitivity (3), diminishing cardiac efficiency (4), and contributing to myocardial lipotoxicity (5–7). Enhanced lipolysis also contributes to body weight loss and cardiac cachexia, which are linked with a particularly poor outcome in HF (2,8–10). Increased adrenergic-fatty acid load in HF has been suggested as a potential therapeutic target (11–14).

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Mechanisms of increased AT lipolysis in HF patients are incompletely understood. Lipolysis in HF patients is stimulated by increased catecholamines (15) and tumor necrosis factor-α (16). Recently, it was recognized that natriuretic peptides (NPs) are also capable of stimulating lipolysis in

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Abbreviations and Acronyms

ANP = atrial natriuretic peptide
AT = adipose tissue
BNP = B-type natriuretic peptide
DGC = dialysate glycerol concentration
FFA = free fatty acid
HF = heart failure
iBNP = immunoreactive B-type natriuretic peptide
NP = natriuretic peptide

adipocytes (17,18). Lipolytic role of atrial natriuretic peptide (ANP) was first suggested by in vitro experiments (19,20) and subsequently confirmed by in vivo studies by our and other groups (20,21). ANP appears to be a major regulator of lipolysis during prolonged exercise (22) in healthy subjects, surpassing the contribution of catecholamines (14,23). ANP is also responsible for the regulation of residual lipolysis in situations of pharmacological beta-adrenergic blockade (2,23). Much less is known about the role of B-type natriuretic peptide (BNP) in lipolysis regulation. Importantly, no studies have examined the lipolytic role of BNP in HF patients, despite important links between NP system activation, enhanced lipolysis, and possible progression of HF.

BNP is released from the heart as inactive pro-$BNP_{1–108}$ in response to increased wall stress (6,9,24) and is then cleft into the bioactive form $BNP_{1–32}$ by specific proteases (9,10,14,17). Despite the fact that circulating immunoreactive BNP (iBNP) is typically increased in HF and serves as a hallmark of the disease (25,26), the functional effects of BNP on target tissues are influenced by abnormal processing/decreased generation of bioactive BNP species (active-BNP deficiency) or by attenuated tissue response (BNP resistance). Which of these 2 mechanisms prevail in AT of HF patients is currently unknown; however, this question can be addressed by examining in vivo lipolytic responses by the administration of exogenous bioactive $BNP_{1–32}$. BNP-mediated lipolysis would be attenuated if the latter mechanism prevails or preserved if the former mechanism is operational.

The aim of this study was to examine the effects of $BNP_{1–32}$ on regulation of lipolysis in vivo and to compare healthy subjects with advanced HF patients. We used the unique technique of microdialysis, which allows tissue delivery of pharmacological substances into subcutaneous AT with simultaneous sampling of extracellular fluid for metabolite analyses.

Methods

Subjects. Men with symptomatic HF (n = 10) were recruited at the Cardiology Department of IKEM, Prague, Czech Republic. HF was defined by Framingham criteria as a history of HF hospitalization, documented systolic dysfunction (left ventricular ejection fraction <35%), and persistent symptoms (New York Heart Association functional class of II or higher for >3 months). Importantly, we excluded patients with diabetes mellitus and hemodynamic instability, infection, severe renal failure or anemia (creatinine >300 μmol/l, hemoglobin <100 g/l), endocrine disease, restrictive or hypertrophic cardiomyopathy. HF etiology was coronary artery disease in 50% and nonischemic in the rest. All patients were treated with furosemide (54 ± 8 mg/day), 9 with beta-blockers and 7 with angiotensin-converting enzyme inhibitors or angiotensin receptor antagonists. Healthy male controls (n = 13), free of any disease or medication, were recruited by advertisement. All subjects were nonsmokers. The study was approved by the local ethics committee, and all subjects signed informed consent.

Clinical measurements. Symptoms were quantified using a Minnesota Living With Heart Failure Questionnaire, body composition was assessed using dual-energy X-ray absorptiometry (Lunar Prodigy, GE Healthcare, Waukesha, Wisconsin), and skinfold thickness was measured with Best's calipers (nondominant triceps, biceps, subscapular, and supra-iliac region). Patients were weighed (Omron HBF510W, Tokyo, Japan), underwent cardiac ultrasonography (Vivid7, GE Healthcare), and their height was measured with a stadiometer. For biochemical tests, venous blood samples were obtained after an overnight fast and plasma was stored at −80°C until analysis. An oral glucose tolerance test was performed on a separate day (75 g of glucose/300 ml of water), with sampling of venous blood at 0, 30, 60, and 120 min. All variables were collected within ±5 days from the microdialysis experiment.

Microdialysis protocol. Microdialysis is a mini-invasive method evaluating AT metabolism in vivo (27). Semipermeable membrane probes are perfused (with or without pharmacological substances) at low flow rates until equilibrium is established between interstitial tissue fluid and dialysate solution in the probe. Glycerol released from adipocytes after triglyceride hydrolysis is used as a lipolysis marker. Microdialysis perfusions with 2 different concentrations of $BNP_{1–32}$ (0.1 μM and 10 μM) were based on previous in vitro data (28) showing maximum lipolytic response with 10 μM and ~50% of maximum effect with a 0.1-μM concentration (2,8,29,30). The subjects entered the research unit at 8:00 AM after an overnight fast. All heparin was withheld for more than 24 h before tests. At 8:30 AM, 4 microdialysis probes (20,000-kDa cutoff, CMA Microdialysis, Stockholm, Sweden) were inserted in the subcutaneous abdominal AT. The probes were connected to a microperfusion pump (Harvard Apparatus, Les Ulis, France) and perfused with Ringer's solution (Baxter, Prague, Czech Republic). Ethanol (1.7 g/l) was added to the perfusate to estimate changes in the blood flow, as described previously (9,10,14). The first probe was perfused with Ringer's solution (control probe), the second probe with 10 μM norepinephrine (Leciva, Prague, Czech Republic), the third probe with 10 μM of human recombinant $BNP_{1–32}$ (nesiritide-Noratak, Janssen-Cilag, Baar, Switzerland) and the fourth probe with 0.1 μM $BNP_{1–32}$. The study consisted of 80-min baseline sampling and 60 min of pharmacological stimulation. Dialysate samples were collected at 20-min intervals at a flow rate of 1 μl/min

Table 1 Anthropometric and Clinical Parameters of HF Patients and Control Group

	HF Patients	Control Group	p Value*
Age, yrs	56.0 ± 3.0	48.0 ± 1.5	0.016
NYHA functional class	3.0 ± 2.8	1 ± 0	<0.001
MLHFQ score	55 ± 7	0.8 ± 0.8	<0.001
Systolic/diastolic BP, mm Hg	102 ± 8/67 ± 6	113 ± 10/79 ± 7	0.05/0.013
Heart rate, beats/min	76.3 ± 4.6	71.9 ± 1.7	0.34
Anthropometric variables			
Body mass index, kg/m^2	25.8 ± 1.1	28.0 ± 0.7	0.041
Waist circumference, cm	96.6 ± 2.8	100.0 ± 1.8	0.284
Skinfold thickness, mm	32.9 ± 5.1	41.1 ± 4.3	0.192
Fat mass, DEXA, kg	25.6 ± 2.6	25.7 ± 1.7	0.905
Lean mass, DEXA, kg	59.4 ± 3.0	62.4 ± 1.5	0.552
Echocardiography			
LV end-diastolic diameter, mm	75.5 ± 3.1	50.7 ± 1.3	<0.001
LV ejection fraction, %	23.6 ± 1.1	60.0 ± 0.0	<0.001
RV dysfunction grade (0–4)	1.5 ± 0.3	0 ± 0	<0.001
Mitral regurgitation grade (0–4)	2.2 ± 0.4	0.7 ± 0.2	0.008

Values are mean ± SD. *p value for comparison of HF and control groups (Mann-Whitney test). Skinfold thickness is a sum of 4 skinfolds.
BP = blood pressure; DEXA = dual-energy X-ray absorptiometry; HF = heart failure; LV = left ventricular; MLHFQ = Minnesota Living With Heart Failure Questionnaire; NYHA = New York Heart Association; RV = right ventricular.

throughout the experiments. The dialysate glycerol concentration (DGC) was measured as a marker of lipolysis.

To evaluate the relative recovery of microdialysis probes (a parameter describing permeability characteristics of the membrane), 4 probes were submerged in a vial containing 0.1 μM $BNP_{1–32}$ dissolved in saline solution. After 3-h equilibration, probes were perfused with saline solution and 0.1% albumin at flow rates 0.5, 1, 2, 3.5, and 5 μl/min. $BNP_{1–32}$ was measured in dialysate and in surrounding $BNP_{1–32}$ solution and log-transformed concentrations were plotted against perfusion rates to calculate the relative recovery at each perfusion rate. This experiment demonstrated a relative recovery of 20 ± 2% for $BNP_{1–32}$ at perfusion rate 1 μl/min.

Biochemical analysis. The iBNP concentrations were measured by chemiluminescent microparticle immunoassay (Architect BNP, Abbott Laboratories, Abbott Park, Illinois), insulin by IRMA assay (Immunotech, Prague, Czech Republic), FFA by immunoturbidimetry (Wako, Richmond, Virginia), and glycerol by Randox Glycerol Kit (Randox Laboratories Ltd., Antrim, United Kingdom).

Statistical analysis. Statistical analysis was performed using SPSS version 13.0 for Windows (SPSS Inc., Chicago, Illinois). The response of AT to pharmacological perfusions was evaluated in the HF and control groups separately using Student's paired *t* test. Differential responses to $BNP_{1–32}$, norepinephrine, or Ringer's solution perfusion between groups were evaluated using an ordinary *t* test. Between-group differences in anthropometric and biochemical variables (Tables 1 and 2) were evaluated using the Mann-Whitney test. Associations between variables were investigated using the Spearman method. Data are presented as mean ± SEM unless stated otherwise. A level of $p \leq 0.05$ was considered statistically significant.

Table 2 Biochemical and Insulin Sensitivity Parameters of HF Patients and Control Group

	HF Group	Control Group	p Value*
BNP, pg/ml	793.2 (549.5–1,838.6)	27.6 (19.9–36.3)	<0.001
BNP, pmol/l	174.5 (120.9–404.5)	6.1 (4.4–8.0)	
Fasting glucose, mmol/l	5.2 ± 0.1	5.5 ± 0.1	0.064
Fasting insulin, mIU/l	8.9 ± 1.6	9.4 ± 1.2	0.595
HOMA-IR	2.1 ± 0.4	2.4 ± 0.3	0.645
HbA_{1c}, %	4.2 ± 0.1	3.7 ± 0.1	0.006
Oral glucose tolerance test			
Glucose AUC, mmol/l/120 min	6,056 ± 3,525	6,057 ± 2,229	0.268
Insulin AUC, mIU/ml/120 min	869.6 ± 146.6	785.6 ± 118.7	0.910
$Glucose_{120\ min}$, mmol/l	6.6 ± 0.5	4.8 ± 0.5	0.025
$Insulin_{120\ min}$, mIU/l	46.5 ± 9.3	19.6 ± 5.2	0.064

Values are median (interquartile range) or mean ± SEM. *p value for comparison of heart failure and control groups (Mann-Whitney test).
AUC = area under the curve; BNP = B-type natriuretic peptide; HbA_{1c} = glycosylated hemoglobin A1c; HF = heart failure; HOMA-IR = homeostasis model assessment of insulin resistance.

Results

Spontaneous lipolysis. Spontaneous lipolysis at baseline was evaluated by analyzing DGC in all samples obtained throughout the last hour of a baseline period. HF patients had higher spontaneous lipolytic rate by 19% than control subjects (189.0 ± 37.3 μmol/l vs. 152.1 ± 35.1 μmol/l, $p < 0.01$) (Fig. 1A). Spontaneous lipolysis (DGC in a control probe) remained unchanged in healthy volunteers, but decreased by 22% in the HF group ($p = 0.01$) during the experiment period.

Response to $BNP_{1–32}$ and norepinephrine. Compared with baseline DGC, the perfusion with 10 μM $BNP_{1–32}$ increased lipolysis by 2.8 ± 0.5-fold and 3.2 ± 0.3-fold (both $p < 0.05$) in control and HF subjects, respectively. The increment in lipolysis induced by 10 μM $BNP_{1–32}$ was similar between groups ($p = 0.61$). The lipolytic response to the lower $BNP_{1–32}$ concentration (0.1 μM) differed between HF and control subjects ($p = 0.02$); lipolysis increased by 17% ($p = 0.056$) in HF subjects, but remained unchanged in the control group ($p = 0.39$). No differences were observed between the HF and control groups ($p = 0.35$) in norepinephrine-induced lipolysis (1.7 ± 0.4-fold and 1.7 ± 0.2-fold increase in controls and HF, $p = 0.04$ and $p = 0.06$, respectively). Analysis of absolute differences in DGC (in μmol/l) after $BNP_{1–32}$ perfusions revealed a trend toward a higher response in HF than in control subjects both at low and high $BNP_{1–32}$ concentrations (by 23% and 41%, respectively, $p = 0.1$ and $p = 0.07$, respectively). Data are summarized in Figures 2 and 3.

Determinants of spontaneous lipolysis and response to $BNP_{1–32}$. Differential regulation of lipolysis was observed between HF and control groups. Spontaneous DGC was associated with adiposity and insulin resistance evaluated as the area under the plasma insulin curve ($AUC_{insulin}$) during the oral glucose tolerance test ($r = 0.67$, $p = 0.01$ and $r = 0.88$, $p < 0.01$) in the control group, whereas these associations were absent in the HF subjects ($r = 0.31$, $p = 0.5$ and $r = -0.52$, $p = 0.18$). No associations were observed between basal lipolytic rate and plasma iBNP in the HF or control group ($r = 0.41$, $p = 0.24$ and $r = 0.5$, $p = 0.1$, respectively) (Fig. 4A). However, lipolytic response to 10-μM $BNP_{1–32}$ perfusion was inversely related to fat mass (%) in the HF group ($r = -0.90$, $p < 0.01$), but no relationship with fat mass or other investigated variables (fasting plasma insulin, homeostasis model assessment of insulin resistance, INS_{120}) was observed in the control group (Fig. 4B).

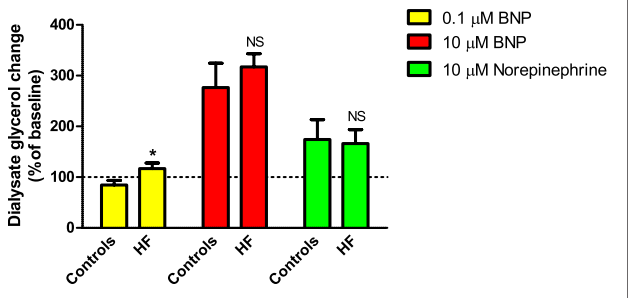

Figure 2 Dialysate Glycerol Concentrations After Pharmacological Stimulation

Values were calculated as the percentage of change in dialysate glycerol concentration at time 140 min over averaged values of spontaneous lipolysis obtained at time points (0 to 80 min). Differences between control and heart failure (HF) groups were analyzed using a *t* test. *$p < 0.05$. BNP = B-type natriuretic peptide.

Changes in AT blood flow and AT cyclic guanosine monophosphate production during $BNP_{1–32}$ administration. Blood flow in AT was evaluated using the ethanol concentration outflow/inflow ratio, as described previously (14,31). No differences were observed in the HF and control groups at baseline or after 10-μM $BNP_{1–32}$ perfusion, which induced vasodilation in both groups. In contrast, norepinephrine administration led to vasodilation in control subjects only (Fig. 1B). Additionally, after the 10-μM $BNP_{1–32}$ perfusion

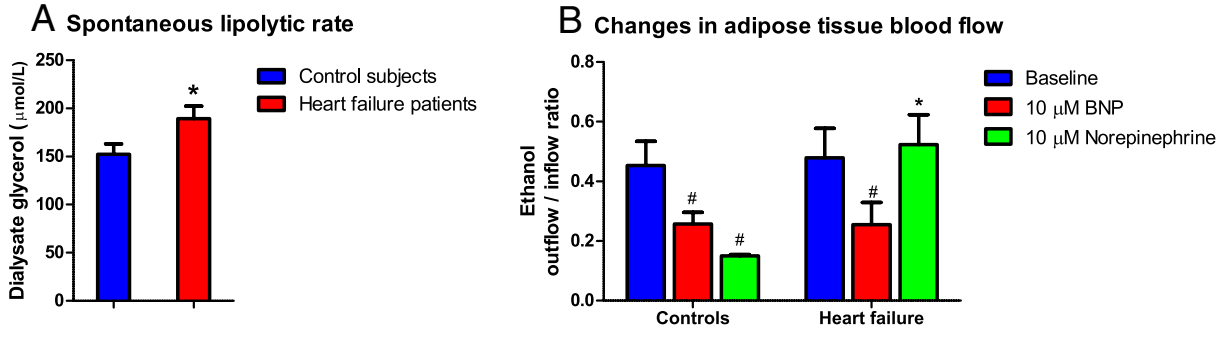

Figure 1 Spontaneous Adipose Tissue Lipolysis and Blood Flow in Control and Heart Failure Subjects

(A) Dialysate glycerol concentrations reflecting baseline lipolytic rate in adipose tissue. **(B)** Changes in ethanol outflow/inflow concentration ratio reflecting adipose tissue blood flow at baseline and after pharmacological stimulation (10 μM B-type natriuretic peptide $[BNP]_{1–32}$ and 10 μM norepinephrine). A paired *t* test was used to compare values before and after perfusions, and an ordinary *t* test was used when comparing differences between groups. *$p < 0.05$ between groups, $N = 5$; #$p <$ 0.05 versus baseline.

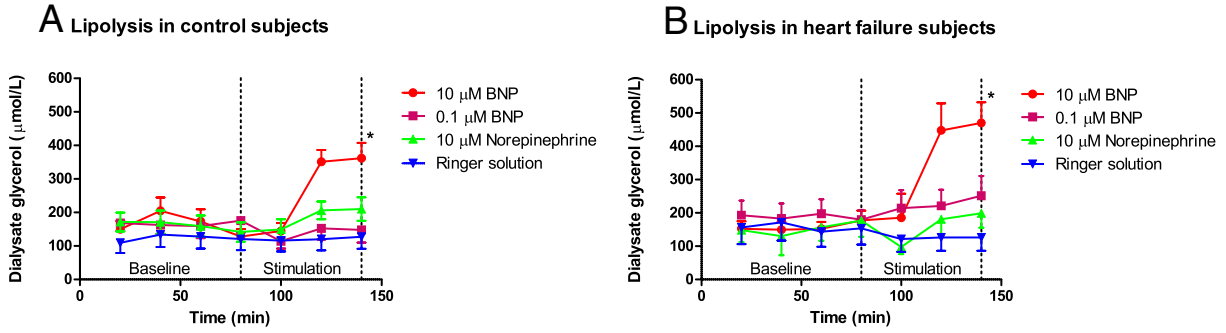

Figure 3 Dialysate Glycerol Concentrations in Individual Probes During Pharmacological Perfusions

Dialysate glycerol concentrations after stimulation with B-type natriuretic peptide $[BNP]_{1–32}$ or norepinephrine. *$p < 0.05$, paired *t* test. **(A)** Control subjects. **(B)** Heart failure subjects.

in a separate group of 4 healthy volunteers, we observed a 43.2 ± 12.8-fold increase ($p < 0.05$) in AT cyclic guanosine monophosphate concentration that was associated with a concomitant increase in DGC ($r = 1$, $p < 0.001$). These results indicate that cyclic guanosine monophosphate–dependent lipolytic pathway is activated in adipocytes during BNP administration.

Discussion

We present several new findings concerning the regulation of lipolysis in AT in HF. In nondiabetic advanced HF patients, spontaneous lipolysis is enhanced compared with body composition–matched healthy controls. In both groups, administration of $BNP_{1–32}$ leads to a strong lipolytic response. Moreover, the responsiveness of AT to $BNP_{1–32}$ is not attenuated, but rather increased in HF, and it is inversely proportional to body fat mass.

Increased spontaneous lipolysis in HF. Humoral factors including NPs (23), catecholamines (14,32), insulin (33), and drugs (e.g., beta-blockers) contribute to lipolysis regulation in HF. The finding of increased spontaneous lipolysis in HF is consistent with a report of increased plasmatic FFA

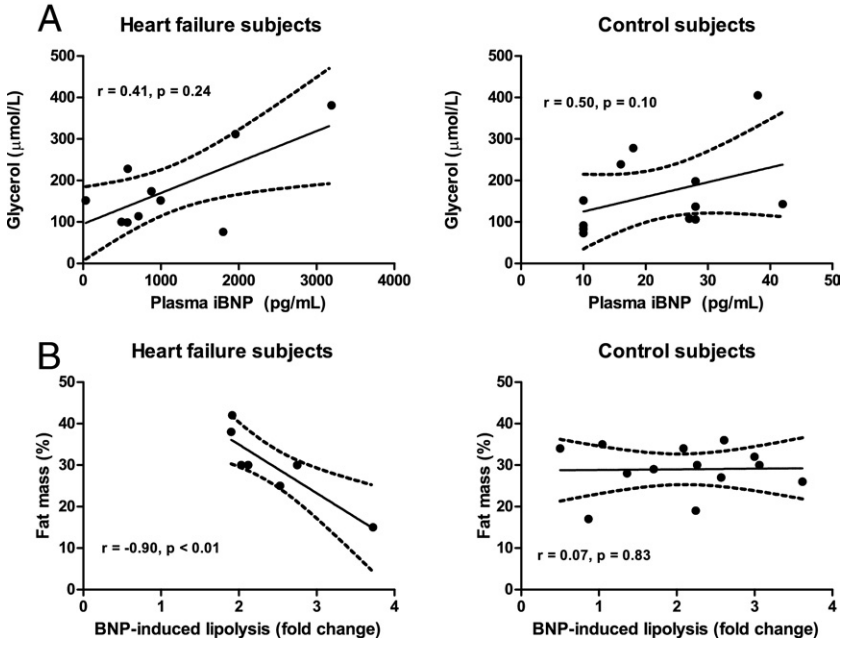

Figure 4 Associations Between Lipolysis Regulation and the BNP System in Control and HF Subjects

Association between plasma immunoreactive B-type natriuretic peptide (iBNP) levels and spontaneous lipolysis **(A)**. Association between lipolytic response to 10-μM B-type natriuretic peptide $(BNP)_{1–32}$ perfusion and fat mass (percentage of body weight) **(B)**. HF = heart failure.

flux in the HF state (1). Lipolysis is physiologically inhibited by insulin, and altered insulin action thus regulates lipolytic rate. Indeed, insulin resistance is frequently observed in nondiabetic HF patients (30). Furthermore, higher plasma insulin levels during the oral glucose tolerance test (representing a dynamic test of glucose metabolism) were associated with greater spontaneous lipolysis in the HF group but not in the control group in our study, indicating that insulin resistance and the attenuated antilipolytic effect of insulin might contribute to greater lipolysis in HF.

$BNP_{1–32}$ induced strong lipolytic effects in both control and HF subjects. The effect of bioactive $BNP_{1–32}$ on AT lipolysis of both groups is in line with previous experiments using local, systemic, or in vitro administration of ANP (14,34,35). The magnitude of lipolysis induced by 10 μM $BNP_{1–32}$ is comparable to that observed after administration of 10 μM ANP (14); however, because the subjects in this study differ from the previous in several cofounders (e.g., body composition, age) further $BNP_{1–32}$ studies are needed to directly compare in vivo lipolytic potency of ANP and BNP. Importantly, because the effects of catecholamines are attenuated in HF by beta-blocker therapy, NPs might be even more important regulators of lipolysis in HF subjects than in control subjects. Despite the fact that HF is often associated with resistance of vascular and renal tissues to the effects of NPs due to multiple mechanisms (36), we did not observe diminished lipolysis induced by $BNP_{1–32}$ in the HF group, suggesting that NP resistance in HF is not ubiquitous in all tissues.

Heightened AT responsiveness to $BNP_{1–32}$. A perfusion with 2 concentrations of $BNP_{1–32}$ allowed us to partly evaluate the BNP sensitivity of AT. Although the response was similar in both groups with 10-μM perfusion, lipolysis activation with 0.1 μM was higher in HF subjects, indicating increased sensitivity of AT to BNP. How then can most of the HF patients maintain their stable fat mass, regardless of a large increase in iBNP? This contradiction could be explained by the notion that bioactive BNP species are actually deficient in HF subjects (36). Mass spectrometry analysis showed that circulating iBNP in HF patients contains mostly biologically inactive BNP pro-forms (20,37). Here we show higher AT sensitivity to $BNP_{1–32}$ despite elevated circulating iBNP and thus provide indirect in vivo support for the hypothesis of active BNP deficiency in HF. Additionally, the absence of an association between iBNP and spontaneous lipolysis in our study further supports this hypothesis.

Interestingly, we observed an inverse association between $BNP_{1–32}$-induced lipolysis and fat mass in HF. In obese subjects, lower lipolytic responsiveness to NPs and therefore lower FFA flux may translate into fewer detrimental effects on cardiac function (5–7) and could underlie a "paradoxical" association between increased fat mass and better HF prognosis (38). Increased NP clearance receptors in AT of obese subjects (39) or the effects of a high-fat diet (40) can explain lower lipolytic $BNP_{1–32}$ responses in obese subjects (40). Furthermore, the ability of BNP to regulate whole-body energy homeostasis (41) via enhanced mitochondrial biogenesis and lipid oxidation in skeletal muscle (40) might also modify plasma FFA levels; however, it remains unclear how these mechanisms are operational in HF. Subsequently, AT might become more sensitive to $BNP_{1–32}$ as body fat is decreasing (e.g., during the weight loss or in cardiac cachexia), further perpetuating the vicious cycle of weight loss.

Effects of $BNP_{1–32}$ on AT blood flow. Because changes in AT blood flow affect DGC, we evaluated AT blood flow during perfusions. Neither AT blood flow at baseline nor the vasodilatory response to 10 μM $BNP_{1–32}$ differed between groups. No effect of norepinephrine on AT blood flow was observed in the HF group, whereas vasodilation occurred in control subjects.

Study limitations. Our work has identifiable limitations. First, it was limited to advanced HF in middle-aged men without diabetes and the number of examined subjects is rather small. Second, concentrations of $BNP_{1–32}$ used in microdialysis perfusions were substantially higher than physiological levels in AT, and it is thus possible that using lower doses would provide additional information in a more physiological range. Third, the HF group was somewhat older than control subjects. Because aging is typically associated with diminished lipolysis (33,42), our results might underestimate the lipolysis of HF compared with younger controls. Future studies using systemic $BNP_{1–32}$ infusion at more physiological concentrations are needed to extend our findings, help to delineate the real cause-and-effect relationship, and describe other endocrine and metabolic changes induced by BNP (e.g., changes in adipokine levels and substrate use). Additionally, studies comparing patients with different HF severity across a range of adiposity and insulin resistance will provide important and clinically relevant information.

Conclusions

Our observations suggest that the lipolytic rate is increased in patients with HF. Furthermore, induction of lipolysis by $BNP_{1–32}$ is increased in HF patients compared with control subjects, which suggests that BNP might play an important role in excessive FFA mobilization and related metabolic abnormalities including cardiac cachexia.

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Key Words: B-type natriuretic peptide ■ free fatty acids ■ heart failure ■ insulin resistance ■ lipolysis.